

(10)



Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number:

0 192 033
A2

(12)

EUROPEAN PATENT APPLICATION

(21) Application number: 86100279.8

(22) Date of filing: 10.01.86

(51) Int. Cl.⁴: C 12 N 15/00

C 12 P 21/02, A 61 K 39/118

C 12 Q 1/68, G 01 N 33/569

(30) Priority: 14.01.85 US 692001

(43) Date of publication of application:
27.08.86 Bulletin 86/35(84) Designated Contracting States:
AT BE CH DE FR GB IT LI LU NL SE(71) Applicant: CHIRON CORPORATION
4560 Horton Street
Emeryville California 94608(US)(71) Applicant: The Board of Trustees of the University of
Washington

Seattle Washington(US)

(72) Inventor: Agabian, Nina
3 Burnett Avenue N., No. 5
San Francisco California 94131(US)(72) Inventor: Stephens, Richard
2715 W. Blaine
Seattle Washington 98199(US)(72) Inventor: Kuo, Cho-Chou
6531 29th Avenue N.E. Seattle
Washington 98115(US)(72) Inventor: Mullenbach, Guy T.
309 63rd Street No. C, Oakland
California 94618(US)(74) Representative: Glawe, Delfs, Moll & Partner
Patentanwälte
Postfach 162 Liebherrstrasse 20
D-8000 München 26(DE)(64) *Chlamydia* major outer membrane protein.

(57) Methods and compositions are provided for the production of a polypeptide which is immunologically cross-reactive with a naturally-occurring major outer membrane protein (MOMP) of *Chlamydia trachomatis*. A DNA construct including a replication system recognized by *E. coli*, and an MOMP gene under the transcriptional control of a β -galactosidase promoter and terminator is provided.

Recombinant phage λ gt11/L2/33 was deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, on January 10, 1985 and granted accession no. 40157. L2 B9-F DNA was deposited at the American Type Culture Collection on December 31, 1985, and granted accession no 40217.

EP 0 192 033 A2

polynucleotide fragment capable of binding a DNA or RNA sequence characteristic of one or more species of Chlamydia trachomatis, and a detectable label bound to the single-stranded polynucleotide.

art of

5

25. A polynucleotide probe as in claim 24, wherein the sequence of the single stranded polynucleotide fragment is substantially homologous or complementary to at least 12 contiguous bases as set forth in Appendix B hereto.

10

ma
ions

lting
us,

15

20

ach
d on
ovar
r

25

s,
ajor
is
ized

30

35

fic
he

various MOMP's generally ranges from about 38kD to 45kD. The serovars display varying antigenic complexity, with certain serovars eliciting broad cross-reactivity with others in the same biovar, while other serovars display little or no such cross-reactivity.

Vaccines utilizing purified and unpurified preparations of intact Chlamydia trachomatis have been prepared and tested on monkeys. While successful protection against subsequent challenge with the same chlamydial serovar was achieved, it was found that heterologous serovar challenge resulted in more severe pathology than that experienced by controls who had not been immunized. In human trials, immunization with the vaccines afforded significant protection against the serovar of the vaccine for up to two years, but hypersensitivity resulted from infection with heterologous serovars.

2. Description of the Relevant Art

The nature of the major outer membrane protein and its relation to the biovars and serovars of Chlamydia trachomatis are discussed in Grayston and Wang (1975) J. Infect. Dis. 132:87-105; Stephens et al. (1982) J. Immunol. 128:1083-1089; and Caldwell et al. (1981) Infect. Immun. 31:1161-1176. Inhibition of infectivity of Chlamydia trachomatis by both anti-chlamydial antisera and monoclonal antibodies has been demonstrated. Caldwell and Perry (1982) Infect. Immun. 38:745-754; and Clark et al. (1982) Infect. Immun. 38:1273-1278. Vaccine trials conducted with intact chlamydial elementary bodies are reported by Collier (1961) Lancet 1:795-800; Wang et al. (1967) Amer. J. Ophthal. 63:1615-1630; and Woolridge et al. (1967) Amer. J. Ophthal. 63:1645-1653. The cloning and expression of a gene encoding a 74,000 dalton chlamydial antigen in E. coli is reported by Stephens et al. (1983) Abstracts Annual Meeting American Society

of Microbiology, B29, p. 35. Stephens et al. failed to obtain expression of a major outer membrane protein. Wenman and Lovett (1982) Nature 296:68-70, report the expression of a 19,000 dalton Chlamydia trachomatis polypeptide. The polypeptide does not appear to be involved in the major outer membrane protein. Allan et al. (1984) Infect. Immun. 45:637-641, recently reported the cloning of the major outer membrane protein gene. Nano et al. (1985) Infec. Immun. 45:637-641 report the sequencing of the first 25 N-terminal amino acids of the major outer membrane protein and the cloning of at least a portion of the gene. An immunoassay for the detection of Chlamydia trachomatis antigen is described in U.S. Patent No. 4,497,899.

15 SUMMARY OF THE INVENTION

Polypeptide compositions having immunological activity corresponding to that of a major outer membrane protein (MOMP) of Chlamydia trachomatis are produced by expressing a chimeric DNA construct comprising a polynucleotide encoding at least a portion of the MOMP under the regulatory control of a regulatory system recognized by a unicellular expression host. The MOMP polynucleotide may code for the entire protein or for a fragment thereof, and may be expressed in conjunction with another structural gene to yield a fused translation product. Such polypeptide compositions will be characterized by the presence of non-interfering amounts of substances derived from the expression, which presence may be used to distinguish the polypeptides of the present invention from the natural polypeptides. The polypeptide compositions of the present invention are useful as substitutes for the naturally-occurring MOMP's of Chlamydia trachomatis, particularly as immunological reagents, e.g., in serological assays to detect the presence of antibodies in blood, the immunogenic substance in vaccines, and the like. The

MOMP polynucleotides will also be useful as labelled probes for diagnostics.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

5 Methods and compositions are provided for the efficient expression of polypeptides demonstrating immunological activity analogous to that of a major outer membrane protein (MOMP) of Chlamydia trachomatis. By analogous immunological activity, it is meant that, when administered to a vertebrate, the polypeptides
10 will elicit an immunological response which is cross-reactive with antibodies elicited by administration of the natural MOMP. The present invention employs a DNA construct including a MOMP polynucleotide or fragment thereof encoding a
15 polypeptide under the transcriptional and translational control of suitable regulatory sequences. MOMP polypeptides free from other chlamydial antigens are obtained by expressing the MOMP polynucleotides in a unicellular host other than Chlamydia trachomatis.
20 The MOMP polynucleotides of the present invention may be initially derived from any of the chlamydial serovars and may be employed in a natural or modified form. MOMP nucleotides having a sequence corresponding to an intact natural MOMP gene will
25 usually be employed when it is desired to express the entire MOMP, although it will be possible to alter the sequence for a desired purpose, e.g., to conform to the codon bias of the expression host (as discussed below), or to create or delete restriction sites, so long as
30 the amino acid sequence is not significantly altered. Shorter DNA sequences corresponding to portions of the MOMP gene will be employed when it is desired to produce only a fragment of the natural proteins. Usually, such sequences will encode for an epitopic
35 region(s), comprising at least 27 bp, usually at least 36 bp, preferably at least 45 bp, and may be much longer.

It will sometimes be desirable to express the MOMP polynucleotide together with other gene(s) in order to provide fused translation products having desirable properties. For example, when producing low molecular weight MOMP polypeptide fragments (below about 5kD), it may be desirable to fuse the MOMP polypeptide to an immunogenic carrier, e.g., tetanus toxoid or hepatitis B surface antigen. It is also possible to fuse MOMP fragments from more than one serovar to each other and/or a gene expressing a suitable immunogenic carrier. Conveniently, the MOMP polynucleotide will be inserted in proper reading frame with the fused gene and under the regulatory control of the regulatory system of the fused gene. Recovery of the gene product may be facilitated by employing a secretory protein as the fused gene product, as described below.

The MOMP polypeptides may be glycosylated, partially glycosylated, or unglycosylated, depending on the nature of the expression host. Generally, prokaryotes such as E. coli will provide no glycosylation of the translated MOMP gene products, while yeast and mammalian cell culture will provide partial or substantial glycosylation. Thus, it will be possible to vary the final MOMP product by appropriate selection of the expression host.

The MOMP polynucleotide may be synthetic or natural, or combinations thereof. A natural MOMP gene (or a portion thereof) may be obtained by preparing a Chlamydia trachomatis genomic library and screening for the presence of the MOMP gene. Screening may be accomplished using antibodies for the gene product or using labelled DNA probes specific for the polynucleotide. Both methods are exemplified in the Experimental section hereinafter. Suitable antibodies are commercially available or may be prepared from purified MOMP obtained from Chlamydia trachomatis by

well known techniques. Suitable DNA probes may be obtained based on the amino acid sequence of the MOMP, or based on the polynucleotide sequence which is reported hereinafter for the MOMP of the L₂ serovar (see Appendix B). Conveniently, the λ gt11/L2/33 clone which has been deposited in connection with this patent application may be labelled and used as a screening probe. A specific method for selecting a clone expressing the MOMP of the L₂ serovar from a Chlamydia trachomatis genomic library is set forth in the Experimental section hereinafter. This method can be modified to allow for selection of MOMP gene(s) from other serovars.

Synthetic polynucleotide sequences encoding for at least a portion of the MOMP gene of Chlamydia trachomatis may also find use, either alone or in combination with the naturally-occurring sequences. Coding for the synthetic sequences may be based on either the reported amino acid sequences for the MOMP's or on the polynucleotide sequences which are determined from the MOMP genes by known techniques. When used for preparing polypeptides as immunological reagents or as vaccines, it is usually desirable that the synthetic nucleotide fragment code for an oligopeptide corresponding to an epitopic site of the natural MOMP. Often, such epitopic sites may be inferred from the folding rules of Chou and Fasman (1974) Biochemistry 13:211-222, in conjunction with an analysis of hydrophobic and hydrophilic regions of the protein as taught by Hopp and Woods (1981) Proc. Natl. Acad. Sci. USA 78:3824-3828. Alternatively, the DNA sequences encoding the polypeptide regions which react with particular monoclonal antibodies may be identified by the high-density phage procedure described by Nunberg et al. (1984) Proc. Natl. Acad. Sci. USA 81:3675-3679. The oligopeptide may then be screened for eliciting Ab cross-reactive with the naturally-occurring MOMP.

A number of techniques are available for synthesizing short, single-stranded DNA fragments, e.g., the phosphoramidite method described by Beaucage and Carruthers (1981) Tetrahedron Lett. 22:1859-1862.

5 A particularly useful adaptation of the method of Beaucage and Carruthers is reported by Warner et al. (1984) DNA 3:401-411. Using the method of Urdea et al., single-stranded DNA fragments having a length of up to 100 bases may be synthesized, and double-stranded
10 DNA fragments may be formed by annealing and ligating a plurality of single-stranded fragments under appropriate conditions. Alternatively, the complementary strand may be added using DNA polymerase with an appropriate primer sequence.

15 When preparing synthetic MOMP polynucleotides, it may sometimes be desirable to modify the natural nucleotide sequence. For example, it will often be preferred to use codons which are preferentially recognized by the desired host. When
20 employing a yeast host, codons which appear at high frequency in the structural genes encoding the yeast glycolytic enzymes may be employed. In some instances, it may be desirable to further alter the nucleotide sequence to create or remove restriction sites to
25 increase stability or to substitute one or more amino acids in the resulting polypeptide. Such changes may be made to enhance the immunogenicity of the polypeptide, facilitate conjugating the polypeptide to a carrier protein, or the like. It may also be
30 desirable to add amino acids to the N-terminus or C-terminus of the polypeptide, where such additional amino acids provide for a desired result.

To produce a desired MOMP polypeptide, the MOMP polynucleotides will be incorporated into DNA
35 constructs capable of being introduced into a desired expression host, usually either a prokaryotic host or eukaryotic host, such as yeast. Such DNA constructs

will include the MOMP polynucleotide encoding the polypeptide product, transcriptional and translational initiation regulatory sequences joined to the 5'-end of the polynucleotide, and transcriptional and translational termination regulatory sequences joined to the 3'-end of the polynucleotide. The DNA constructs will usually also include a replication system recognized by the expression host to allow for self-replication, and will often include other functional sequences such as markers allowing for selection of transformed hosts, additional replication systems, secretory leader and processing signal sequences, and the like. The replication system, however, is not necessary since the DNA construct may allow for integration into the host genome. Integration is facilitated by providing short DNA fragments on either side of the MOMP polynucleotide, which fragments are homologous to a desired location in the host genome.

The transcriptional initiation regulatory sequences will include a promoter region recognized by the expression host. For E. coli hosts, the lac promoter, lambda P_L or P_R , or the β -galactosidase promoter, as exemplified in the Experimental section hereinafter, are suitable. For yeast hosts, suitable promoters include those involved with the enzymes in a yeast glycolytic pathway, such as the promoters for alcohol dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase, triose phosphate isomerase, phosphoglucisomerase, phosphofructokinase, and the like. By employing these promoters with other regulatory sequences, such as enhancers, operators, and the like, and using a host having an intact regulatory system, one can regulate the expression of the MOMP polypeptide by a number of techniques, such as varying the carbon source, e.g., replacing glucose with galactose; varying the concentration of a nutrient,

e.g., acid phosphatase, or changing the temperature with a temperature sensitive promoter or regulatory system.

The transcriptional termination regulatory sequence will include a terminator, preferably a terminator balanced with the promoter to provide proper transcription. Conveniently, the terminator which is naturally found with the promoter may be employed. In the exemplary embodiment described in the Experimental section hereinafter, the MOMP polynucleotide is inserted between the β -galactosidase promoter and terminator within the β -galactosidase structural gene so that a fusion product is formed.

Enhanced yields of the polypeptides of the present invention may be obtained by employing DNA constructs which include a secretory leader and processing signal sequence to effect secretion of the gene product in yeast. The use of such secretory leader and processing signal sequences will be particularly effective with polypeptides below about 40 kilodaltons, more usually below about 30 kilodaltons, although it is expected that the system will function with polypeptides equal to the length of the whole MOMP, i.e., ranging from 38 to 45 kilodaltons. The secretory leader and processing signal sequences will normally be derived from naturally-occurring DNA sequences in yeast which provide for secretion of a polypeptide. Such polypeptides which are naturally secreted by yeast include α -factor, α -factor, acid phosphatase, and the like. If desired, the naturally-occurring sequence may be modified, for example, by reducing the number of lys-arg pairs in α -factor which define the processing site (while retaining at least one pair), or by reducing the length of the secretory leader sequence (while retaining sufficient length to provide for secretion) or by introducing point mutations, deletions or other

modifications which facilitate manipulation, e.g., introducing restriction recognition sites.

Conveniently, the secretory leader and processing signal sequence may be joined to the MOMP polynucleotide by providing appropriate cohesive ends on the polynucleotide fragment, by use of appropriate adaptor molecules, or a combination of both. A portion of the structural gene for the secretory protein may be left in the final DNA construct when it is desired to produce a fused translation product, as discussed above.

Polypeptides of the present invention may also be recovered intracellularly as follows. After the transformed cell culture has reached a high density, the cells will be separated, typically by centrifugation, lysed, and the MOMP polypeptides isolated and purified by various techniques, such as extraction, affinity chromatography, electrophoresis, dialysis, and combinations thereof.

The MOMP polypeptides may also be prepared by conventional solid-phase synthesis techniques, such as those described by Merrifield (1963) J. Am. Chem. Soc. 85:2149-2156. Such solid-phase techniques are suitable for preparation of polypeptide fragments of up to about 50 to 100 amino acids, or more. Generally, however, as the length of the polypeptide increases above 25 amino acids, the difficulty in the synthesis increases and the desirability of employing a solid-phase synthesis technique diminishes.

The polypeptides of the present invention, and fragments thereof, may be employed in a variety of ways. The polypeptides can be employed both as labelled and unlabelled reagents in various immunoassays, bioassays, and the like, for the detection of Chlamydia trachomatis or antibodies to Chlamydia trachomatis in a biological sample, e.g., serum. Suitable labels include radionuclides,

enzymes, fluorescers, chemilumescers, enzyme substrates or co-factors, enzyme inhibitors, particles, dyes, and the like. Such labelled reagents may be used in a variety of well known assays, such as

5 radioimmunoassays, enzyme immunoassays, e.g., ELISA, fluorescent immunoassays, and the like. See, for example, U.S. Patent Nos. 3,766,162; 3,791,932; 3,817,837; 3,996,345; and 4,233,402. Polypeptides of the present invention may also find use in vaccines

10 against infection by Chlamydia trachomatis. Larger polypeptides, having a molecular weight exceeding about 5,000 daltons, may be used without further modification. Smaller haptens (i.e., those below about

15 immunogenic carrier in order to elicit the desired immune response. Suitable immunogenic carriers include tetanus toxoid and hepatitis B surface antigen. It will be possible to link short DNA fragments expressing the MOMP polypeptides to genes expressing proteins from

20 other pathogenic organisms or viruses. In this way, the resulting fused proteins may provide immunity against more than one disease.

In preparing a vaccine, the polypeptides will normally be incorporated in a physiologically

25 acceptable medium, such as water, physiological saline, phosphate buffered saline, and the like. The vaccine may be administered intravenously, intraarterially, subcutaneously, intraperitoneally, or the like. The amount of immunogen employed per dose will be about 5

30 to 10 micrograms, if liquid, in a volume of about 0.25 to 1 ml, and may be administered repeatedly at about 2 to 4 week intervals, usually not more than 2 or 3 times.

The polynucleotides of the present invention

35 may be employed as labelled polynucleotide probes suitable for screening biological samples for the presence of various strains of Chlamydia trachomatis.

Probes comprising DNA or RNA from conserved regions of the MOMP gene may be employed for detecting a broad range of Chlamydia, while probes comprising regions of the MOMP gene specific for a particular strain may be employed to identify that strain. The polynucleotide sequences in such probes will typically be at least 12 nucleotides, more typically 16 or more nucleotides. Conveniently, the nucleotide fragment may be synthesized based on the sequence set forth in Appendix A, hereinafter.

Suitable labels include radionuclides, heavy metals, organic ligands, and the like, which allow for detection in conventional assays. Biological samples will be prepared in a conventional manner, e.g., by lysing the Chlamydia to release the nucleic acids.

EXPERIMENTAL

The following experiments are offered by way of illustration, not by way of limitation.

MATERIALS AND METHODS

20 Reagents

DNase, RNase, endonuclease restriction enzymes, T4 ligase, kinase, DNA Polymerase I, and EcoRI methylase were obtained from Bethesda Research Laboratories. Nitrocellulose was obtained from Schleicher and Schuell. Peroxidase conjugated anti-mouse, anti-rabbit, and peroxidase anti-peroxidase (PAP) sera were obtained from Cappell. Proteinase K, isopropylthiogalactoside (IPTG), and 4-chloro-1-naphthol were from Sigma Chemical Co. Phage packaging mix was obtained from Amersham. CNBr-activated Sepharose[®]-4B was obtained from Pharmacia.

Bacterial Strains

35 E. coli Y1088, Y1089, Y1090, and BNN 97 were obtained from R. Young and R. Davis (Stanford University). For C. trachomatis, two trachoma strains, B/TW-5/OT, and C/TW-3/OT, and one LGV strain,

L₂/434/Bu, were grown in HeLa 229 cells and Renografin purified as described by Kuo et al. (1977) in: "Nongonococcal Urethritis and Related Infections," Hobson and Holmes, eds., Am. Soc. Microbiol. pp.

5 176-185.

Antibodies

Polyvalent antiserum to C. trachomatis was obtained from rabbits immunized with purified LVG (L₂ serovar) organisms that were grown in chick embryo yolk sacs. Anti-E. coli reactivities in this antiserum were removed by passage through a Sepharose[®]-4B column derivitized with an E. coli lysate. For this purpose, approximately 20mg of DNase and RNase treated lysate of induced BNN 97 were coupled to 1mg of CNBr-activated Sepharose[®]-4B according to the manufacturer's instructions. The development, specificities, and ascites production of monoclonal antibodies specific for C. trachomatis have been previously reported (Stephens et al. (1982) J. Immunol. 128:1083).

20 Insertion of Chlamydial DNA into λ gt11

Chlamydial DNA was isolated from cell extracts of serovars L₂, B, and C by proteinase K treatment (65 μ g/ml, 45°C, 1 hr.) and solubilization in 1% sodium dodecyl sulfate (SDS). Following phenol extraction, the preparations were treated with 50 μ g/ml RNase (60°C, 30 min.), phenol/chloroform extracted, and ethanol precipitated. Standard procedures were used for enzymatic reactions and for isolation of λ phage DNA (Molecular Cloning Maniatis et al. Cold Springs Harbor Lab., 1982). Chlamydial DNA from serovar L₂ (150 μ g) was partially digested with DNase I as previously described (Ruther et al. (1982) Proc. Natl. Acad. Sci. USA 79:6852). Digested DNA was fractionated in a 1.25% agarose gel, and 500-2000 base pair fractions were collected on Whatman DE-81 paper and eluted as previously described (Dretzen et al. (1981) Annals of Biochem. 112:295). After treatment with DNA

polymerase I, the DNA was methylated with EcoRI methylase, and 2 μ g of this preparation were ligated to phosphorylated EcoRI linkers with T4 ligase. These fragments were then cleaved with EcoRI endonuclease and fractionated on a Sepharose[®] G-150 column. Chlamydial DNA fractions were pooled and ethanol precipitated, and 20ng of the chlamydial DNA were ligated to 1 μ g of EcoRI cleaved λ gt11. The EcoRI site is located within the β -galactosidase gene under the regulatory control of the β -galactosidase promoter and terminator. The ligated DNA was packaged into phage according to the manufacturer's instructions. Phage were plated and amplified in E. coli Y1088, and approximately 2×10^5 recombinant phages were obtained.

15 Screening of Recombinant Phages

E. coli Y1090 was infected with recombinant phage preparations that resulted in approximately 10^4 plaque forming units (PFU) per 150mm plate. Plates were initially incubated at 42°C until small plaques became visible (approx. 5 hrs.). Plates were then overlaid with IPTG saturated nitrocellulose disks and incubated an additional 2 hrs. at 37°C. The nitrocellulose disks were carefully removed from the plates, rinsed in phosphate buffered saline (PBS) (pH 7.4) to remove any residual agar, and blocked in PBS containing 5% bovine serum albumin (BSA) for 60 min. at 37°C to prevent subsequent nonspecific adsorption of protein. The disks were incubated with monoclonal antibodies (1:1000 dilution in PBS containing 0.05% Tween[®]-20) for 2 hr., at room temperature or overnight at 4°C. The disks were washed for 1 hr. with 6 changes of PBS-Tween[®] and incubated with peroxidase-conjugated, anti-mouse antibody (1:2000) for 1 hr. at room temperature, followed by a 1 hr. incubation with peroxidase anti-peroxidase (PAP) (1:2000 dilution). The disks were then washed with 6 changes of PBS-Tween[®] followed by 2 changes of PBS. The immune reactions

were detected by adding 0.5mg/ml of 4-chloro-1-naphthol and 0.001% H_2O_2 in PBS and agitated for 5-15 min.

Plaques showing positive reactions were selected, plated at low densities, and reassayed with antibody.

- 5 This process was repeated until all plaques were reactive.

Analysis of Proteins by SDS-PAGE and Immunoblotting

- Lysogens were produced from selected λ gt11 recombinants by infecting E. coli Y1089 as previously described by Young and Davis (1983) Proc. Natl. Acad. Sci. USA 80:1194. Lysates from induced recombinant lysogens were prepared, and 20 μ g aliquots were electrophoresed on 7.5% or 10% SDS-polyacrylamide gels (SDS-PAGE) according to Laemmli (1970) Nature 227:680.
- 10 The proteins in some gels were stained with Coomassie brilliant blue, while those from other gels were electrophoretically transferred to nitrocellulose for immunoblotting, as described by Towbin (1979) Proc. Natl. Acad. Sci. USA 76:4350-4354. Following
- 20 electrophoretic transfer, nitrocellulose sheets were blocked in 5% BSA and probed with either a 1:1000 dilution of rabbit polyvalent anti-C. trachomatis antiserum or mouse ascites containing high titered monoclonal antibody. Immune reactions were detected as
- 25 described above for the screening of recombinant plaques, except that the PAP step was omitted. Prestained molecular weight standards were: myosin (200,000), phosphorylase B (92,500), BSA (68,000), ovalbumin (43,000), chymotrypsinogen (25,700),
- 30 lactoglobulin (18,400), and cytochrome C (12,300) (Bethesda Research Laboratories).

Characterization of λ gt11/L2/33 Insert DNA

- λ gt11/L2/33 insert DNA was obtained from EcoRI digests of the recombinant phage and separated on agarose gels. For dot blot hybridization, 32 P-labelled
- 35 insert DNA was reacted with lysates of C. trachomatis serovars A/G-17/OT, B/TW-5/OT, Ba/AP-2/OT, C/TW-3/OT,

D/UW-3/Cx, E/UW-5/Cx, F/UW-6/Cx, G/UW-57, H/UW-43/Cx, I/UW-12/Ur, J/UW-36/Cx, K/UW-53/Cx, L₁/440/Bu, L₂/434/Bu, L₃/404/Bu, C. psittaci strain Mn, and HeLa 229 host cells. Lysates were prepared from approximately 10 μ g of each chlamydial strain by proteinase K digestion (1mg/ml in 10mM Tris, pH 8.5, and 1mM EDTA) for 1.5 hr. at 37°C. Samples were made to 0.2N NaOH, heated to 100°C for 5 min., and placed on ice. The NaOH was neutralized with one volume of cold 0.2M acetic acid, followed by 0.5 volume of cold 20XSSC. The samples were filtered through nitrocellulose sheets and the sheets were washed with 6XSSC, air dried and baked 3 hr. at 80°C. The sheets were probed with ³²P-labelled λ gt11/L2/33 insert at 65°C by standard procedures (Molecular Cloning supra.) Southern blots of BamHI-digested C. trachomatis DNA and endonuclease restriction mapping of the λ gt11/L2/33 insert were performed by standard procedures (Molecular Cloning supra.).

20 Insertion of Chlamydial DNA into λ 1059

A library of chlamydial genomic DNA was produced in the bacteriophage lambda 1059 system, which cloning system was described by Karn et al. (1980) Proc. Natl. Acad. Sci. USA 77:5172-5176. C. trachomatis L₂ DNA was randomized by partial digestion with endonuclease restriction enzyme Sau3A or cleaved with BamHI and ligated to BamHI digested vector. Ligated DNA was packaged in vitro, as described by Sternberg et al. (1977) Gene 1:255-280, and plated in E. coli Q359 for screening as described in Karn et al. (1980) supra. Phages were plated in E. coli Q359 at densities of approximately 3 x 10³ plaque forming units per 150mm plate. The plates were overlaid with nitrocellulose disks and the disks containing plaque adsorbed DNA were air dried and baked 3hr at 80°C. The disks were probed with ³²P labelled λ gt11/L2/33 insert DNA at 60°C by standard procedures (Molecular Cloning,

supra). Several plaques that produced strong signals were picked and reassayed as above until all plaques from a clone were uniformly reactive. DNA was isolated from the selected phage recombinants by standard procedures (Molecular Cloning, supra). Two clones were mapped by endonuclease restriction analysis and Southern blotting by standard procedures (Molecular Cloning, supra). Both λ 1059 recombinants had more than one BamHI insert, however, bands with identical gel mobilities were identified which were not shared with bands from the vector, and some of these bands hybridized to the insert DNA probes in Southern blots. This process permitted mapping of contiguous endonuclease restriction sites that flanked the location of the homolog to the λ gt11/L2/33 insert. The map obtained by endonuclease restriction analyses was verified by generating subclones of specific fragments in a plasmid vector (pUC 18), and predicted cross-hybridizations between these clones and with the λ 1059 recombinants were observed in Southern blots. Fragments that included the putative coding region and flanking regions were used for DNA sequencing.

RESULTS

Detection of Chlamydial Antigens

DNA obtained from C. trachomatis serovar L₂ was partially digested with DNase I and inserted into the bacteriophage vector λ gt11. The resulting plaques were transferred to nitrocellulose for the direct detection of C. trachomatis-specific antigens. Polyvalent anti-L₂ rabbit serum detected seven plaques that produced strong immune reactions from among the 2×10^4 recombinant plaques assayed. The positive plaques were replated at low densities and screened with polyvalent antiserum.

After plaque purification, the seven recombinants were tested with a pool of monoclonal antibodies. The monoclonal antibody pool consisted of

four antibodies (2C1, 2G1, 2H2, AE11) that each bind a mutually exclusive MOMP determinant (Stephens et al. (1982) supra.) One of the clones, designated λ gt11/L2/33, reacted with the pool of antibodies, while the other six recombinant clones did not. Subsequently, λ gt11/L2/33 was tested with each of over 15 monoclonal antibodies representing species-, subspecies-, and type-specific anti-chlamydial reaction patterns. The specificities of the antibodies and their reaction pattern with λ gt11/L2/33 are presented in Table 1. The reaction pattern demonstrated that λ gt11/L2/33 was producing a polypeptide that displays species-, subspecies-, and type-specific epitopes of the chlamydial MOMP. The lack of reaction of λ gt11/L2/33 to antibodies not reactive with the L_2 serovar was expected since the recombinant was derived from serovar L_2 DNA. Two antibodies (AE11 and 3H10) that do react with native L_2 MOMP did not react with the polypeptide expressed by λ gt11/L2/33 using this plaque assay. The two antibodies, however, gave positive reactions with λ gt11/L2/33 expressed polypeptide in immunoblotting.

25

30

35

Table 1

	Monoclonal Antibody No.	Serovar Specificities	Reaction with λ gt11/L2/33	
	2C1, IH8	all serovars	+	---
5	AE11	all serovars except C	-*	---
	3H10	A,B,D,E,F,G,H,K,L1,L2,L3	-*	---
	KG5	B,D,E,F,G,H,K,L1,L2,L3	+	---
	DA10	B,D,E,G,F,L1,L2,L3	+	---
	2G3	B,D,E,K,L1,L2,L3	+	---
10	2G1	B,F,G,H,K,L2,L3	+	---
	3H1, 2IIE3	B,D,E,L1,L2	+	---
	JC8	B,D,G,F,L2	+	---
	FE10	E,G,F,L2	+	---
	JG1	B,D,E,L2	+	---
15	2H2, 2H5	L2	-	---
	1B7, DD1	B	-	---
	2B1	C,J	-	---
	FC2	F	-	---
	JG9	D	-	---

20

* Positive reaction obtained by immunoblotting.

Analysis of Recombinant Fusion Polypeptides

25 E. coli lysogens were prepared for each of the positive λ gt11 clones to provide a source of fusion polypeptides for analysis. Lysates obtained from induced lysogens were assessed by Coomassie blue stained PAGE gels and by immunoblotting of the proteins that were electrophoretically transferred from PAGE

30 gels to nitrocellulose. The molecular weights of these fusion proteins were estimated to range from 132,000 to 146,000.

Immunoblot analysis of PAGE gels using polyvalent rabbit anti-L₂ revealed that each of the

35 seven clones produced strong reactions in the plaque assay. The λ gt11/L2/33 product stained most intensely, while the products from two other recombinants stained

very faintly. Immunoblot analysis was also performed with the monoclonal antibodies. Of the seven recombinants, only λ gt11/L2/33 reacted with monoclonal antibodies as expected from the results on the plaque assays with these same antibodies. The monoclonal antibodies that recognized species-specific and subspecies-specific determinants on L_2 chlamydial MOMP reacted strongly with the polypeptide produced by λ gt11/L2/33, while the L_2 type-specific monoclonal antibodies produced negative or equivocal reactions.

5
10

Characterization of λ gt11/L2/33 Insert DNA

DNA from the λ gt11/L2/33 recombinant was isolated, labelled with ^{32}P , and used to probe dot blots of each of the 15 C. trachomatis serovars, the Mn strain of C. psittaci, and HeLa 229 host cells. Reactions were detected with all chlamydiae but not with HeLa 229 host cell DNA. Furthermore, Southern blots of BamHI digests of C. trachomatis DNA obtained from serovars L_2 , B, and C revealed one fragment in each preparation which reacted with ^{32}P -labelled insert DNA from λ gt11/L2/33. The molecular weight of this fragment varied slightly between serovars but was approximately 9.4 kb.

15
20

Preparations of λ gt11/L2/33 insert DNA were obtained from EcoRI digests and separated on agarose gels. The insert was estimated to be about 1.1kb in length with restriction sites for HaeII, HaeIII, HhaI, and XhoI. Restriction sites for AccI, BamHI, BclI, BstEII, EcoRI, EcoRV, PstI, PvuI, SstI, and SstII were not detected.

25
30

The approximately 1.1 kb insert DNA was sequenced by standard techniques, and the sequence is set forth in Appendix A.

Sequencing of λ 1059 Inserts

Lambda 1059 recombinants having 9.2 to 9.8 kb inserts that were shown to be homologous with λ gt11/L2/33 by Southern analysis were used for

35

endonuclease restriction mapping, and additional Southern analyses. Two contiguous fragments (BamHI/EcoRI and EcoRI/EcoRI) were identified, and these contain sufficient base pairs to encode for the L₂ MOMP gene product. These fragments were cloned into M13 for DNA sequencing. The sequence data for a 9.2 kb fragment (designated L2 B9-F DNA) are set forth in Appendix B.

The sequence includes an untranslated region comprising 1287 bases, followed by a 66 base region encoding a 22 amino acid leader sequence. Coding for the MOMP begins at base 67 (amino acid 23) and extends through base number 1182 (amino acid 394). The molecular weight for the MOMP including the leader is calculated to be 42,557 daltons.

The N-terminus of the MOMP was located on the basis of the 25 amino acid N-terminus reported by Nano et al. (1985) supra. Differences in the sequences of the N-terminus reported by Nano et al. and that reported herein are found at amino acid residues 32, 44, and 45, as numbered in Appendix B. These differences may result from differences among the isolates or mistakes in amino acid sequencing.

The sequence set forth in Appendix A corresponds to amino acids 247 through the 3'-terminus in Appendix B, with certain deviations. Bases 36-38 in Appendix A are AGA, corresponding to amino acids GlyGlu, while bases 773-775 in Appendix B are TGT, corresponding to amino acids GlyVal. These deviations are underlined in both Appendices. The DNA sequence corresponding to amino acids 305 through 394 in Appendix B has several deviations from Appendix A which result in a different reading frame for the sequence of Appendix B. Base numbers 174, 181, and 186 in Appendix A were not detected in the λ 1059 clones. Base number 35 in Appendix A is a T, while the corresponding base in Appendix B (in amino acid 357) is a C. Finally, a G is inserted in amino acid 358 and a G is inserted in

amino acid 374 in the sequence of Appendix B. In both Appendices A and B, bases which are inserted or changed relative to the other Appendix are boxed, while deleted bases are indicated by an arrow. Both the DNA and amino acid sequences of Appendix B are believed to be correct.

According to the subject invention, novel recombinant DNA constructs are provided for the expression of a polypeptide having immunological activity corresponding to that of a naturally-occurring major outer membrane protein of Chlamydia trachomatis. Such polypeptides may find use as reagents in the detection of Chlamydia trachomatis or antibodies to Chlamydia trachomatis, and as vaccines against infection by Chlamydia trachomatis in susceptible hosts.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

25

30

35

1 GluPheProLeuAlaLeuLysAlaGlyThrAspGlyGluGlyThrLysAspAlaIle
 GAATTCCTCTTGAATTAAAGCAGGAACAGATGGAGAGAGGGAAGTAAGGATGCCTCT
 CTTAAGGGAGAACTAGAAATTTCTGCTCTTGTCTACCTCTCTGCTCTTGTCTACGGAGA
 1 ecor1, 7 mn1, 13 mbol sau3a, 47 dde1, 51 fok1, 56 mn1,
 61 IleAspTyrHisGluTrpGlnAlaSerLeuAlaLeuSerTyrArgLeuAsnMetPheThr
 ATTGATTACCATGAATGGCAAGCAAGTTTAGCTCTCTCTTACAGACTGAATATGTTCACT
 TAACTAATGGTACTTACCGTTCTGTTCAAATCGAGAGAGAATGTCTGACTTATACAAGTGA
 90 alul, 108 xmn1,
 121 ProTyrIleGlyValLysTrpSerArgAlaSerPheAspAlaAspThrIleArgTyrCys
 CCCTACATTGGAGTTAAATGGTCTCGAGCAAGTTTTGATGCAGACACGATTCTGTTATTGC
 GGGATGTAACCTCAATTTACCAAGCTCGTTCAAACCTACGTCCTGTGCTAAGGATAACG
 143 ava1 xho1, 144 taq1, 168 hinf1, 180 mn1,
 181 LeuSerProLysSerAlaThrThrValPheAspValThrThrLeuAsnProThrIleAla
 GTCAGGCGGAGTTCAGCTACAACCTGTCTTTGATGTTACCACTCTGAACCCAACTATTGCT
 GAGTCTGGCTTCAGTCTGATGTTGACAGAACTACAATGGTGAGACTTGGGTTGATAACGA
 181 dde1, 195 alul,
 241 GlyAlaGlyAspValLysAlaSerAlaGluGlyGlnLeuGlyAspThrMetGlnIleVal
 GGAGCTGGCGATGTGAAAGCTACCGCAGAGGGTCAAGCTCGAGATACCATGCAAATCGTT
 CCTCGACCGCTACACTTTGATCGCGCTCTCCAGTCTGAGCCTCTATGGTACGTTTAGCAA
 243 alul, 258 alul, 263 hha1, 268 mn1, 275 alul,
 301 SerLeuGlnLeuAsnLysMetLysSerArgLysPheSerValLeuGlnAM
 TCCTTGCAATTGAACAAGATGAAATCTAGAAAATTTTCGGTATTGCAGTAGGAACAACCTA
 AGGAACGTTAACTTGTCTACTTTAGATCTTTTAAAGCCATAACGTCATCCTTGTGAT
 325 xba1, 329 xmn1,
 361 TTGTGGATGCAGACAAATACGCATTACAGTTGAGACTCGCTTGATCGATGAGAGAGCTGC
 AACACCTACGTCTGTTTATGCGTAATGTCAACTCTGAGCGAAGTACTCTCTCGACG
 365 fok1, 394 hinf1, 403 mbol sau3a, 404 clal, 405 taq1, 415
alul, 416 bbv fnu4h1,
 421 TCACGTAAATGCACAATTCCGCTTCTAATTAATTGTATAATTTTGTTAACTTTGGCAAG
 AGTGCATTTACGTGTTAAGGCGAAGATTAATTAACATATTAAACAATTTGAAACCGTTC
 481 TTTATCTTTGTTAATAACGTTAATAACACTATCCGTGTTTTCTGGGCTCGACTTCCGGTCCG
 AAATAGAAACAATTATTGCAATTATTGTGATAGGCACAAAGACCCGAGCTGAAGCCAGCC
 523 ban2 hsil sdu1, 527 taq1, 539 ban1, 540 asul ava2,
 541 GTCCAGTTTTTTTTTGCAAAAATTTTTTTTTCTTACTTTTCGATCTCCCTCCTATCTCTCTTA
 CAGGTCAAAAAAACGTTTTTAAAAAAGGAATGAAAGCTAGAGGGAGGATAGAGAGAAT
 577 taq1, 579 mbol sau3a, 585 mn1,
 601 CAACAAAATCTAAAATTTCTCTAAAAGAAGATTGCATAAAAGGCCTCTTTCCAGTACTAT
 GTTGTTTTAGATTTTAAAGAGATTTTCTTCTAACGTATTTTCCGGAGAAAGGTCATGATA
 627 mbol1, 641 hae1 stu1, 642 hae111, 644 mn1, 653 sca1, 65
 4 rsal,
 661 ATCGGTCTACTTGAGCGCGCCCGTAGCTCAATGGTAGAGCTGTAGCCTTCCAAAGCTACCG
 TAGCCAGATGAAGTCCCGCGGGCATCGAGTTACCATCTCGACATCGGAAGGTTTCGATGGC
 665 acc1, 675 hha1, 676 tac1, 677 hha1, 685 alul, 698 alul,
 713 alul, 718 hpa1,

APPENDIX B

GGATCCTCACCCTCTTCATAAGCAGCAATGCATTCTCTTAGGTTTCCTAACTCCCTCGT
AATTTTTCTATGTTCTTCTGCGCTAATAGGTCGACATACCCCAACAAATCAGCTACTG
TGGGCCCTTCAGGATAATGTCTGCGAACCAGGATTCGACATGCAATCCTGGCCAATCC
TTCTCCACCATCTTGAGCCTTAAAAAAGTAGGCTCAGATACATTAGGCTCTGAAGGATGT
AAGGTACAGACCCCTAATACAGAGCTTTGGCATGGATCGTATCTTCAACGAAATCACGG
TCCATATGCAGCTCTTGAGCCAAAAAATCTTGCAAATTTTTTAATGTAATCCTTACGAA
CAGGAACAAGCCTCTTATTCCCTTGCTCATCTGTATGCCAAACACGGCTCGGAATATCA
CGTATCGCTCGATAAGAGATCCCCACATTATATTGTAAACGTTTTTCTGCCAGCGTTT
ACCAAAACGATCACATACTCCTGCGGCAACAAATGCTCAGGAACACTTCTACGCTGAG
GACGATAAGCTTCTTCTTCTTTTTCTCATGCTGACACACAGCAACATGCCATATACCC
AAAGTGATGATAGACAAAGCAATAATAATCCACAGTAGTCTGTAGCCTTTTCTGG
CACAGAAAGTGGGGTGGCTGGTTTTCTTTTCATATATGGTTAGTTTAATCTGTTTTAT
TGGTCGACCGTTTAAAAACACTTTCTTTGTAGTAATAAAACGATTTCTATCAAAACAA
ATTCTTAGATTTTCTTACAAAATCTCTCTTTTCTTTTAGCCAAACCCCCATCTTGA
GCTATTCCAAACACAAAAATCTTAGGTTTTGGAAATTAACAACCTCATAAAAATTGAAC
GTTTTGTAAATTAACCAAAACCTCTCATCTCAACAATCAACATATTGCCAATATGGC
TTTTGCTCTCGGTTTCAGAGCGATTTTTTTTGGCAAAACCAAGAACATAAAACATAAAA
AGATATACAAAAATGGCTCTCTGCTTTATCGCTAAATCAGGAGGCGCTTAAGGGCTTCT
TCCTGGGACCAACGTTTTTCTTATCTTCTTTACGACAATAAGAAAATTTTGTATGGCT
CGAGCATTGAACGACATGTTCTCGATTAAAGGCTGCTTTTACTTGCAAGACATTCTCAG
GCCATTAATTGCTACAGGACATCTTGTCTGGCTTTAACTAGGAGGCACTGCCGCCAGAA

AAAGATAGCGAGCACAAAGAGAGCTAATTATACAATTTAGAGGTAAGA														1 Met Lys ATG AAA
Lys	Leu	Leu	Lys	Ser	Val	Leu	Val	Phe	Ala	Ala	Leu	Ser	Ser	Ala
AAA	CTC	TTG	AAA	TGG	GTA	TTA	GTG	TTT	GCC	GCT	TTG	AGT	TCT	GCT
Ser Ser Leu Gln Ala Leu Pro Val Gly Asn Pro Ala Glu Pro Ser														20
TCC	TCC	TTG	CAA	GCT	CTG	CCT	GTG	GGG	AAT	CCT	GCT	GAA	CAA	AGC
Leu Met Ile Asp Gly Ile Leu Trp Glu Gly Phe Gly Gly Asp Pro														30
CTT	ATG	ATC	GAC	GGA	ATT	CTA	TGG	GAA	GCT	TTC	GGC	GGA	CAT	CCT
Cys Asp Pro Cys Thr Thr Trp Cys Asp Ala Ile Ser Met Arg Met														40
TGC	GAT	CCT	TGC	ACC	ACT	TGC	TGT	GAC	GCT	ATC	AGC	ATG	CCT	ATG
Gly Tyr Tyr Gly Asp Phe Val Phe Asp Arg Val Leu Gln Thr Asp														50
GGT	TAC	TAT	GCT	GAC	TTT	GTT	TTC	GAC	GCT	GTT	TTG	CAA	ACA	GAT
Val Asn Lys Glu Phe Gln Met Gly Ala Lys Pro Thr Thr Ala Thr														60
GTG	AAT	AAA	GAA	TTC	CAA	ATG	GCT	GCC	AGG	CCT	ACA	ACT	GCT	ACA

	Asn	Ala	Ala	Ala	Pro	Ser	100	Thr	Cys	Thr	Ala	Arg	Glu	Asn	Pro
GGC	AAT	GCT	GCA	GCT	CCA	TCC	ACT	TGT	ACA	GCA	AGA	GAC	AAT	CCT	
Ala	Tyr	110	Arg	His	Met	Gln	Asp	Ala	Glu	Met	Phe	120	Thr	Asn	Ala
GCT	TAC	GGC	CGA	CAT	ATG	CAG	GAT	GCT	GAG	ATG	TTT	ACA	AAT	GCT	
Ala	Tyr	Met	Ala	Leu	Asn	Ile	130	Trp	Asp	Arg	Phe	Asp	Val	Phe	Cys
GCT	TAC	ATG	GCA	TTG	AAT	ATT	TGG	GAT	CGT	TTT	GAT	GTA	TTC	TGT	
Thr	Leu	140	Ala	Thr	Ser	Gly	Tyr	Leu	Lys	Gly	Asn	150	Ser	Ala	Ser
ACA	TTA	GGG	CCC	ACC	AGT	GGA	TAT	CTT	AAA	GGA	AAT	TCA	GCA	TCT	
Phe	Asn	Leu	Val	Gly	Leu	Phe	160	Gly	Asp	Asn	Glu	Asn	His	Ala	Thr
TTC	AAC	TTA	GTT	GGC	TTA	TTC	GGA	GAT	AAT	GAG	AAC	CAT	GCT	ACA	
Val	Ser	170	Asp	Ser	Lys	Leu	Pro	Asn	Met	Ser	Leu	180	Asp	Gln	Ser
GTT	TCA	GAT	AGT	AAG	CTT	GTA	CCA	AAT	ATG	AGC	TTA	GAT	CAA	TCT	
Val	Val	Glu	Leu	Tyr	Thr	Asp	190	Thr	Thr	Phe	Ala	Trp	Ser	Ala	Gly
GTT	GTT	GAG	TTG	TAT	ACA	GAT	ACT	ACT	TTT	GCT	TGG	AGT	GCT	GGA	
Ala	Arg	200	Ala	Ala	Leu	Trp	Glu	Cys	Gly	Cys	Ala	Thr	210	Gly	Ala
GCT	CGT	GCA	GCT	TTG	TGG	GAA	TGT	GGA	TGC	CCG	ACT	TTA	GGA	GCT	
Ser	Phe	Gln	Tyr	Ala	Gln	Ser	220	Lys	Pro	Lys	Val	Glu	Glu	Leu	Asn
TCT	TTC	CAA	TAC	GCT	CAA	TCC	AAG	CCT	AAA	GTC	GAA	GAA	TTA	AAC	
Val	Leu	230	Cys	Asn	Ala	Ala	Glu	Phe	Thr	Ile	Asn	Lys	240	Lys	Gly
GTT	CTC	TGT	AAC	GCA	GCT	GAG	TTT	ACT	ATC	AAT	AAG	CCT	AAA	GGA	
Tyr	Val	Gly	Gln	Glu	Phe	Pro	250	Leu	Asp	Leu	Lys	Ala	Gly	Thr	Asp
TAT	GTA	GGG	CAA	GAA	TTC	CCT	CTT	GAT	CTT	AAA	GCA	GGA	ACA	GAT	
Gly	Val	260	Thr	Gly	Thr	Lys	Asp	Ala	Ser	Ile	Asp	Tyr	270	His	Glu
GGT	GTC	ACA	GGA	ACT	AAG	GAT	GCC	TCT	ATT	GAT	TAC	CAT	CAA	TGG	
Gln	Ala	Ser	Leu	Ala	Leu	Ser	280	Tyr	Arg	Leu	Asn	Met	Phe	Thr	Pro
CAA	GCA	AGT	TTA	GCT	CTC	TCT	TAC	AGA	CTG	AAT	ATG	TTC	ACT	CCC	
Tyr	Ile	290	Gly	Val	Lys	Trp	Ser	Arg	Ala	Ser	Phe	Asp	300	Ala	Thr
TAC	ATT	GGA	GTT	AAA	TGG	TCT	CGA	GCA	AGT	TTT	GAT	GCA	GAC	ACG	
Ile	Arg	Ile	Ala	Gln	Pro	Lys	310	Ser	Ala	Thr	Thr	Val	Phe	Asp	Val
ATT	CGT	ATT	GCT	CAG	CCG	AAG	TCA	GCT	ACA	ACT	GTC	TTT	GAT	GTT	
Thr	Thr	320	Leu	Asn	Pro	Thr	Ile	Ala	Gly	Ala	Gly	Asp	330	Val	Lys
ACC	ACT	CTG	AAC	CCA	ACT	ATT	GCT	GGA	GCT	GGC	GAT	GTC	AAA	GCT	
Ser	Ala	Glu	Gly	Gln	Leu	Gly	340	Asp	Thr	Met	Gln	Ile	Val	Ser	Leu
AGC	GCA	GAG	GCT	CAG	CTC	GGA	GAT	ACC	ATG	CAA	ATC	GTT	TCC	TTG	
Gln	Leu	350	Asn	Lys	Met	Lys	Ser	Arg	Lys	Ser	Cys	Gly	360	Ala	Val
CAA	TTG	AAC	AAG	ATG	AAA	TCT	AGT	AGA	AAA	TGT	TGC	GGT	ATT	GCA	GTA
Gly	Thr	Thr	Ile	Val	Asp	Ala	370	Asp	Lys	Tyr	Ala	Val	Thr	Val	Glu
GGA	ACA	ACT	ATT	GTC	GAT	GCA	GAC	AAA	TAC	GCA	GCT	ACA	GTT	GAG	

0192033

380
Thr Arg Leu Ile Asp Glu Arg Ala Ala His Val Asn Ala Gln Phe
ACT CGC TTG ATC GAT GAG AGA GCT GCT CAC GTA AAT GCA CAA TTC

394
Arg Phe OC
CGC TTC TAA TTAATTGTATAATTTTGTAAACTTTGCCAAGTTTATCTTTGTTAATA
ACGTTAATAACACTATCCGTGTTTCTGGGCTCGACTTCGGTCCGGTCCAGTTTTTTTTTGC
AAAAATTTTTTTTCTTACTTTTCGATCTCCCTCCTATCTCTCTTACAACAAAATCTAAAT
TTCTCTAAAAGAAGATTGCATAAAAGGCTCTTTCCAGTACTATATCGGTCTACTTGAGG
GCCGCCGTAGCTCAATGGTAGAGCTGTAGCCTTCCAAGCTACCGGTGTCAAGTTCGATTCT
GATCGGGCTCTTTTTTACTCCTGTATGACTCCCAAGTCTGAAATCTGAGCGTCTCTCAAA
TGCTTGTTAACACATAAAAAGAGGAACAAAGCTTGGAACTTTCCTGCAAACTCACTTAA
AAAGAACTATTAGAATCCGGGGCACATTTTGGACACCAGACAGTCCGCTGGAATCCCAAG
ATGAAGCCTTTTATTTTGAAGAAAAAAATGGCCTTTACATCATCGACTTGGCTAAAAC
TTAGGTCAGTTGAAAAAGGCTGTTTCTTGCATTCAAAAACATATCGATCAAGAGAGGTCT
ATTTTTGTTTGTGGAACAAAAAACAAAGCAAAACAGATCATTAGAGAAGCTGCTATCGA
ATGTGGCGAATTC

1

WHAT IS CLAIMED IS:

1. A polypeptide composition capable of eliciting the production of antibodies to a major outer membrane protein (MOMP) of Chlamydia trachomatis, said polypeptide composition including non-interfering amounts of a microorganism other than Chlamydia trachomatis.
2. A polypeptide as in Claim 1, which polypeptide is produced by introducing into the unicellular host a DNA construct including a MOMP polynucleotide.
3. A polypeptide as in Claim 2, wherein the MOMP polynucleotide is at least 27 base pairs in length.
4. A polypeptide as in Claim 2, wherein the MOMP polynucleotide encodes for substantially the entire length of a major outer membrane protein.
5. A polypeptide as in Claim 2, wherein the MOMP polynucleotide is fused to a second structural gene in proper reading frame therewith and is under the regulatory control of the regulatory system of the second structural gene.
6. A polypeptide as in Claim 5, wherein the second structural gene codes for an immunogen.
7. A polypeptide as in Claim 5, wherein the second structural gene is a β -galactosidase gene.
8. A polypeptide as in Claim 1, wherein the unicellular host is Escherichia coli.

9. A polypeptide as in Claim 1, wherein the immunological activity is cross-reactive with Chlamydia trachomatis serovar L₂.

5 10. A DNA construct comprising a MOMP polynucleotide coding for at least a portion of the amino acid sequence of a major outer membrane protein of Chlamydia trachomatis, said DNA fragment being flanked at its 5'-end with transcriptional and
10 translational initiation regulatory sequences and at the 3'-end with transcriptional and translational termination regulatory sequences, wherein at least one of said transcriptional or translational regulatory sequences is not derived from Chlamydia trachomatis.

15 11. A DNA construct as in Claim 10, wherein the MOMP polynucleotide is at least 27 base pairs in length and encodes for an epitopic site of the MOMP.

20 12. A DNA construct as in Claim 10, wherein the MOMP polynucleotide encodes for substantially the entire length of a major outer membrane protein.

25 13. A DNA construct as in Claim 10, wherein the MOMP polynucleotide is fused to a second structural gene in proper reading frame therewith and is under the regulatory control of the regulatory system of the second structural gene.

30 14. A DNA construct as in Claim 13, wherein the second structural gene codes for an immunogen.

35 15. A DNA construct as in Claim 13, wherein the second structural gene is a β -galactosidase gene..

16. A DNA construct as in Claim 6, further comprising a replication system.

17. A DNA construct as in Claim 16, wherein the replication system is a prokaryotic replication system.

5 18. A DNA construct as in Claim 17, wherein the prokaryotic replication system is recognized by Escherichia coli.

10 19. A DNA construct as in Claim 18, wherein the prokaryotic replication system is derived from λ phage.

15 20. A polynucleotide encoding a polypeptide having immunological activity cross-reactive with a major outer membrane protein of Chlamydia trachomatis, said polynucleotide being substantially free of natural flanking regions.

20 21. A polynucleotide as in Claim 20, wherein the major outer membrane protein is from Chlamydia trachomatis serovar L₂.

25 22. A polynucleotide as in Claim 20, having a nucleotide sequence substantially as set forth in Appendix B hereto, or a portion thereof including at least 12 bases.

30 23. An immunoassay for the detection of Chlamydia trachomatis or antibodies to Chlamydia trachomatis in a biological sample, said immunoassay characterized by the use as reagents of the polypeptides of Claim 1, where the polypeptides may be labelled or unlabelled.

35 24. A polynucleotide probe for detecting the presence of Chlamydia trachomatis in a biological sample, said probe comprising a single stranded

polynucleotide fragment capable of binding a DNA or RNA sequence characteristic of one or more species of Chlamydia trachomatis, and a detectable label bound to the single-stranded polynucleotide.

5

25. A polynucleotide probe as in claim 24, wherein the sequence of the single stranded polynucleotide fragment is substantially homologous or complementary to at least 12 contiguous bases as set forth in Appendix B hereto.

10

15

20

25

30

35

